ENHANCED SOYBEAN PLANT GROWTH BY INOCULATION WITH *Bradyrhizobium japonicum* AND *Bacillus* sp.

Jelena Marinković[1], Dragana Bjelić[1], Branislava Tintor[1], Vojin Đukić[1],
Svetlana Balešević-Tubić[1], Duško Marinković[2], Marija Cvijanović[3]
[1]Institute of Field and Vegetable Crops, Maksima Gorkog 30, 21000 Novi Sad, Serbia
[2]Victoria Group, Hajduk Veljko Sq. 11, Novi Sad, Serbia
[3]University of Belgrade, Faculty of Agriculture, Nemanjina 6, 11080, Belgrade-Zemun, Serbia

Abstract

The aim of the experiment was to investigate the effect of strains Bradyrhizobium japonicum and Bacillus sp. on length and dry weight of soybean plants. Moreover, the strains were tested for their ability to produce indole-3-acetic acid and perform phosphate solubilization. The trial was established under semi-controlled conditions. Inoculation of soybean with Bradyrhizobium japonicum and Bacillus sp. increased height and dry weight of the aboveground parts and root of soybean plants. Most variants in which a mixture of Bradyrhizobium japonicum and Bacillus sp. was applied, resulted in significant increase in plant length and higher dry weight of the aboveground parts, compared to variants in which these bacterial strains were applied as single-component inoculants. Results for length and dry weight of the aboveground parts were lowest in uninoculated plants.

Key words: *inoculation, plant dry weight, plant length, soybean*

1. INTRODUCTION

Attaining a sustainable supply of proteins intended for human consumption is a global challenge for industry and science. Soybean (*Glycine maxima*, L.) is one of the most significant cultivated legumes, with steady annual increase of the global cultivation area. Soybean grains are high in protein and, as such, they are used in human and animal nutrition after processing. Great agricultural, economic, and environmental importance of legumes is reflected not only in high-quality composition of grains, but also in the ability of these plants to bind nitrogen from the atmosphere in community with bacteria. This biological process leads to a major reduction in nitrogen fertilizer additions, and significantly reduces pollution linked to nitrogen fertilizer synthesis and spreading (Avice et al. 2011). It also reduces ground water pollution with nitrate and emissions of the greenhouse gas nitrous oxide (Prudent et al. 2015). Bacteria belonging to the genus Bradyrhizobium are of enormous agricultural value since they are able to fix atmospheric N in symbiosis with several leguminous plants, especially soybean. The most important soybean microsymbionts are bacterial species *Bradyrhizobium japonicum* and *Bradyrhizobium elkani* (Ferreira & Hungria 2002). Soybean plants obtain very high proportions of their N from BNF because soybean is an efficient N fixer (Guimaraes et al. 2008).

Lack of the basic mineral elements in soils reduces yield and quality of cultivated plants, which caused a significant increase of mineral fertilizer application in plant production. The production of mineral fertilizers requires high amounts of non-renewable energy sources and substantial economic costs, whereas their application may negatively affect the stability of soil ecosystem. Useful interactions between plants and rhizosphere microorganisms are important determinants of soil fertility. Soil microorganisms have an irreplaceable role in biochemical cycles of organic and inorganic elements in soils, and in maintenance of soil health and quality.

The use of microorganisms with the aim of improving nutrients availability for plants is an important practice and necessary for agriculture (Freitas et al., 2007). During the past couple of decades, the use of plant growth promoting rhizobacteria (PGPR) for sustainable agriculture has increased tremendously in various parts of the world (Das et al., 2013).

Plant growth-promoting rhizobacteria (PGPR) have been shown to enhance plant growth and plant production through a wide range of mechanisms Dodd et al. (2010). There are several ways in which plant growth promoting bacteria can facilitate plant growth, either indirectly or directly (Friesen et al. 2011; Goh et al. 2013). They may: fix atmospheric nitrogen and supply it to plants, synthesize phytohormones such as auxins, cytokinins and gibberellins, solubilize minerals such as phosphorus, making them more readily available for plant growth (Glick 1995; Glick et al. 2007; Bashan & de-Bashan 2010). Bacteria may affect plant growth and development by using anyone or more than one of these mechanisms. Since many plant growth-promoting bacteria possess several of these traits simultaneously, different mechanisms at various times during the life cycle of the plant can be used (Masciarelli et al., 2014).

The most widely studied plant growth-promoting rhizobacteria (PGPR) are the species of the genera *Pseudomonas, Azospirillum, Azotobacter, Klebsiella, Enterobacter, Burkholderia, Bacillus.* (Sakthivel & Karthikeyan, 2012). These bacteria participate in the solubilization of inorganic phosphate, enable biological fixation of atmospheric nitrogen, enhance adoption of other nutrient elements, produce phytohormones (indole-3-acetic acid), and thereby profoundly influence plant growth. (Zaidi et al., 2009)

Implementation of effective microbial strains in the form of microbial fertilizers is the most important factor of increased yield of cultivated plants and the best way to avoid negative effects of mineral fertilizer use. Introduction of new methods and growing systems provide more cost-effective production and technology, which will be widely implemented in the future.

The aim of this study was the characterization of a PGPR and its evaluation for co-inoculation with *B. japonicum* on soybean plants. We evaluated the possibility of growth promotion improvement in soybean through inoculation with *Bradyrhizobium japonicum* and *Bacillus* sp. strains

2. MATERIAL AND METHODS

Investigations were carried out in three stages.

At first stage, bacterial strains of *Bradyrhizobium japonicum*, and *Bacillus* sp. were examined for their ability to produce indole-3-acetic acid (IAA) and perform the solubilization of inorganic phosphate.

The second stage of research included planting of soybean inoculated with the selected strains of *Bradyrhizobium japonicum* and *Bacillus* sp. in semi-controlled conditions.

The third stage included determination of length of the aboveground plant parts, root length, and dry weight of the aboveground plant parts and roots.

The study used *Bradyrhizobium japonicum* and *Bacillus* sp. strains taken from the collection of the Department of Microbial Preparations, Institute of Field and Vegetable Crops, Novi Sad. The study was carried out on the commercial soybean cultivar Proteinka (first maturity group), developed at the Soybean Department, Institute of Field and Vegetable Crops, Novi Sad.

2.1. *Indole-3-acetic acid production and inorganic phosphate solubilization*

2.1.1. Indole-3-acetic acid (IAA) production

For quantitative analysis of IAA production, a 100 µl 24h-old bacterial suspension (standardized to OD_{600} of 0.625) was inoculated in Yeast Extract Mannitol (for *Bradyrhizobium japonicum*) and Meat extract (*Bacillus* sp.) liquid medium, supplemented without and with 250 and 500 µg ml^{-1} of L-tryptophan (as precursor of IAA) and incubated for 48h at standard temperature. Salkowski reagent ($FeCl_3$-$HClO_4$: 2% 0.5 M ferric chloride in 35% perchloric acid) was mixed with the supernatant (2:1 v/v) and intensity of the developed color was measured at 530 nm (Glickman and Dessaux, 1995).

2.1.2. Phosphate solubilization

The ability of isolates to dissolve sparingly soluble inorganic phosphate was determined by spot inoculations on PVK (Pikovskaya medium) (Pikovskaya, 1948) with 0.5% TCP [$Ca_3(PO_4)_2$] and

$FePO_4$ x $2H_2O$. The appearance of clear zones was in positive correlation with the phosphate solubilizing ability. Diameters of the clear zones around the colonies after 5 days of incubation at 28°C were measured and the relative efficacy of P-solubilization was determined according to their values.

2.2. *The experiment on plants in semi-controlled conditions*

The experiment was conducted at the Soybean Department, Institute of Field and Vegetable Crops Novi Sad, at the location Rimski Šančevi. The plants were grown in pots, with a mixture of chernozem and sand in a ratio of 3: 1 used as substrate.

Plants were inoculated with the selected strains of *Bradyrhizobium japonicum* (two strains) and *Bacillus* sp. (four strains) seven days after sowing, while control plants were not inoculated. The liquid inoculum was prepared in an appropriate culture medium. The *Bradyrhizobium japonicum* strains were cultured in YEM (Yeast Extract Mannitol) broth and *Bacillus* sp. strains were grown in nutrient broth (Meat extract). The number of cells in each inoculum was min. $1x10^9$.

Experiment was set as a randomized block design with three replications and 15 variants:

1. Ø – control, without inoculation
2. *Bradyrhizobium japonicum* – strain 1 (R1)
3. *Bradyrhizobium japonicum* – strain 2 (R2)
4. *Bacillus* sp. – soj 1 (B1)
5. *Bacillus* sp. – soj 1 + *Bradyrhizobium japonicum* – strain 1 (B1 + R1)
6. *Bacillus* sp. – soj 1 + *Bradyrhizobium japonicum* – strain 2 (B1 + R2)
7. *Bacillus* sp. – soj 2 (B2)
8. *Bacillus* sp. – soj 2 + *Bradyrhizobium japonicum* – strain 1 (B2 + R1)
9. *Bacillus* sp. – soj 2 + *Bradyrhizobium japonicum* – strain 2 (B2 + R2)
10. *Bacillus* sp. – soj 3 (B3)
11. *Bacillus* sp. – soj 3 + *Bradyrhizobium japonicum* – strain 1 (B3 + R1)
12. *Bacillus* sp. – soj 3 + *Bradyrhizobium japonicum* – strain 2 (B3 + R2)
13. *Bacillus* sp. – soj 4 (B4)
14. *Bacillus* sp. – soj 4 + *Bradyrhizobium japonicum* – strain 1 (B4 + R1)
15. *Bacillus* sp. – soj 4 + *Bradyrhizobium japonicum* – strain 2 (B4 + R2)

After germination, the number of the plants was reduced to six per pot. During the growing season, the plants were exposed to external environmental conditions with optimal additional water supply. The average daily temperature during the experiment was 22.8 ° C, and did not differ significantly from average temperatures for this time of year. Length of the aboveground plant parts and roots were measured between R3 and R5 stages of soybean development, when the plants were at full flowering stage. The plants were dried at 50 ° C for 24 hours, after which dry weights of the aboveground plant parts and roots were measured.

2.3. *Statistical analysis of data*

Statistical processing of data was conducted using Statistica for Windows 10. The differences between the arithmetic treatment means were tested using two-factorial Analysis of Variance, and least significant difference LSD ($p<0.05$) was calculated for each year of study.

3. RESULTS AND DISCUSSION

3.1. *Determination of PGPR trait via phosphate solubilization and IAA production*

Bacterial strains were analyzed for their growth promoting traits, such as phosphate solubilization and IAA production.

Table 1. Determination of phosphate solubilization and IAA production

Strain	Phosphate solubilization $Ca_3(PO_4)_2$		Phosphate solubilization $FePO_4$ x $2H_2O$		Indole-3-acetic acid (IAA) production (µg ml^{-1})
	growth	solubilization	growth	solubilization	
B. japonicum 1 (R1)	+	—	—	—	7.85
B. japonicum 2 (R2)	+	+	+	—	10.80
Bacillus sp. 1 (B1)	+	—	—	—	13.50
Bacillus sp. 2 (B2)	+	+	+	+	15.66
Bacillus sp. 3 (B3)	+	+	—	—	18.20
Bacillus sp. 4 (B4)	+	+	+	+	20.83

Screening of phosphate solubilization activities was done on the basis of clear halo zone formation on Pikovskaya's agar.

Two sources of inorganic phosphate in the culture medium, $Ca_3(PO_4)_2$ and $FePO_4$ x $2H_2O$, were used in the tests of ability of the strains to solubilize inorganic phosphates. All of the tested strains grew on the substrate in which calcium phosphate was the source of inorganic phosphate, and R1 and B1 strains did not show the ability of the solubilization of calcium phosphates. However, when the $Ca_3(PO_4)_2$ is replaced with x $FePO_4$ $2H_2O$, only strains R2, B2 and B4 had the ability to grow. The strain of *Bradyrhizobium japonicum* 2, though it was growing in a medium with FePO4, did not show the ability to solubilize iron (II) phosphate (Table 1).

A number of soil microbes can cause the solubilization of insoluble phosphate, and thereby convert insoluble inorganic phosphates to the plant available forms (Anwar et al., 2014). The formation of organic acid, phosphatase and its function in phosphate solubilisation is entirely known. (Goldstein & Krishnaraj, 2007) Dileep Kumar et al. (2001) also reported that combined inoculation of pea seeds with rhizobial and phosphate solubilizing bacteria increased plant height. Richardson (2000) reported that in soils with low available P, the use of P fertilization represents high cost to farmers. Rodriguez & Fraga (1999) report that *Pseudomonas*, *Bacillus* and *Rhizobium* are among the bacteria with high potential for P solubilization in soil. The main mechanism to P solubilization by bacteria is the release of organic acids that are combined with other metabolites like phytohormones and lytic enzymes (Vassilev et al., 2006).

IAA is one of the most important phytohormones functioning as important signal molecule in the regulation of plant development. (Anwar et al., 2014). The aim of this study was to determine the potency of plant growth-promoting rhizobacteria *Bradyrhizobium japonicum* and *Bacillus* sp. as producers of IAA and their application in soybean production.

In our research, IAA production by *Bradyrhizobium japonicum strains* were 10.8 μg ml^{-1} (R1) and 7.85 μg ml^{-1} (R2), but levels of IAA production were higher by *Bacillus* sp. strains (13.50-20.83 μg ml^{-1}). The highest IAA production was observed by *Bacillus* sp. strain B4 (20.83 μg ml^{-1}) (Table 1).

Some studies suggest that IAA also played an important role in the formation of legume nodule (Hunter, 1994). Fuhrmann (1993), described the diversity of *Bradyrhizobium* strains dividing them into two groups, *B. elkanii* and *B. japonicum*, according to IAA production. Higher production of IAA by the new isolated strain shows that its plant-growth promoting activity includes not only symbiotic nitrogen fixation, but also additional properties such as high production of phytohormones and enhanced enzymatic activities (Masciarelli et al., 2014).

3.2. *The experiment on plants in semi-controlled conditions*

Table 2. Effects of inoculation with *Bradyrhizobium japonicum* and *Bacillus* sp. strains on soybean traits

Inoculation treatments	Height of aboveground plant parts (cm)	Root length (cm)	Dry weight of aboveground plant parts (g $plant^{-1}$)	Dry weight of roots (g $plant^{-1}$)
Ø	32.78 ± 0.30 ef	43.33±1.15 bc	2.69±0.23 g	0.633±0.d
R1	36.75 ±2 .46 bcd	49.00±5.29 abc	3.37±0.28 ef	0.727±0.abcd
R2	35.03 ± 1.41 de	48.33 ± 2.08 abc	3.73 ± 0.25 cde	0.707±0.abcd
B1	33.00 ± 1.30 ef	48.67 ± 1.53 abc	2.93 ± 0.17 fg	0.740±0.abcd
B1 + R1	39.26 ± 1.15 ab	47.00 ± 7.00 abc	3.93 ± 0.40 bcd	0.763±0.abc
B1 + R2	38.91 ± 0.88 abc	48.67 ± 7.09 abc	3.93 ± 0.09 bcd	0.780±0.abc
B2	36.22 ± 0.96 cd	42.00 ± 8.72 c	3.67 ± 0.05 cde	0.623±0.d
B2 + R1	38.81 ± 1.05 abc	48.67 ± 0.58 abc	3.50 ± 0.17 de	0.687±0.bcd
B2 + R2	40.02 ± 0.49 a	48,67 ± 1.53 abc	4.07 ± 0.44 abc	0.657±0.cd
B3	31.78 ± 1.83 f	49.67 ± 2.52 abc	2.99 ± 0.09 fg	0.783±0.abc
B3 + R1	40.22 ± 1.43 a	52.33 ± 8.33 ab	4.39 ± 0.55 ab	0.787±0.ab
B3 + R2	39.93 ± 1.70 abc	50.33 ± 2.52 abc	4.48 ± 0.31 a	0.833±0.a
B4	37.81 ± 2.50 abcd	55.33 ± 8.33 a	4.06 ± 0.49 abc	0.697±0.bcd
B4 + R1	38.99 ± 3.49 abc	49.33 ± 2.31 abc	4.33 ± 0.34 ab	0.707±0.abcd
B4 + R2	38.94 ± 2.96 abc	48.33 ± 9.60 abc	4.49 ± 0.14 a	0.700±0.bcd

*Means followed by the same letter are not statistically different (LSD, $p < 0.05$)

Inoculation of soybean with *Bradyrhizobium japonicum* and *Bacillus* sp. strains had greater impact on the increase in height and dry weight of the aboveground plant parts (Table 2).

Height of aboveground plant parts inoculated with strains R2, B1 and B3, and dry weight of plants inoculated with strains B1 and B3, was not significantly different from the height and weight of control, i.e. non-inoculated plants. For all other treatments, the application of both single-component and two-component inoculants positively influenced the development of aboveground plant parts, and both the height and dry weight of inoculated plants were significantly higher compared with the uninoculated control (Table 2).

On most inoculation treatments, using a mixture of *Bradyrhizobium japonicum* and *Bacillus* sp. strains resulted in significantly greater length and dry weight of the aboveground parts, compared to treatments where the same bacterial strains were applied as single component inoculants. Maximum height of the aboveground parts was observed in the plants inoculated with a mixture of strains B3 + R1 (40.22 cm), and B2 + R2 (40.02 cm), while the lowest aboveground parts were observed in uninoculated plants (32.78 cm) (Table 2).

The two-component inoculation treatments resulted in maximum dry weight of the aboveground parts of soybean plants. Using a mixture of strains B3 + R2 resulted in dry weight of 4.48 g $plant^{-1}$, and inoculation with strains B4 + R2 resulted in dry weight of 4.49 g $plant^{-1}$. The lowest dry weight of the aboveground parts was observed in control - uninoculated plants (2.69 g $plant^{-1}$) (Table 2).

Application of the selected bacterial strains had less effect on the length and weight of the root system (Table 2).

Significantly longer root system compared to control was only observed after treatment with the strain B4 (55.33 cm). Significant differences in root length was not observed between different variants of inoculation. The minimum root length was found after treatment with the strain B2 (42.00 cm). Unlike root length, several inoculation treatments positively affected root dry weight.

The minimum root dry weight was observed in non-inoculated plants (0.633 g $plant^{-1}$) and after treatment with the strain B2 (0.623 g $plant^{-1}$). The highest root dry weight was observed in the plants inoculated with a mixture of strains B3 + R2 (0.833 g $plant^{-1}$) (Table 2).

Studies carried out by Dubey (1996) and Wasule et al. (2007) also clearly revealed that co-inoculation of *Bradyrhizobium* and PGPR microorganisms significantly improved soybean growth and its yield components as compared with the sole application of *Bradyrhizobium.* Considering the benefits to crop growth attributed to PGPR, co-inoculation with these microorganisms might improve plant's performance. This approach is in agreement with modern demands of agricultural, economic and environmental sustainability (Chaparro et al. 2012). Other authors also suggest that in soybean plants, synergism between *Bacillus* and *Bradyrhizobium* in the rhizosphere has been shown to promote plant growth and crop yields (Medeot et al. 2010; Tsigie et al. 2012). Raeipour and Aliasgharzadeh, (2004) also stated that *Bradyrhizobium* bacteria has positive effect on shoot dry weight, and interaction of phosphate solubilizing bacteria and *B. japonicum* was significant on shoot dry weight. Co-inoculation studies with PGPR and *Rhizobium*/*Bradyrhizobium* spp. have been shown to increase root and shoot biomass, nodule dry matter, N2-fixation, and grain yield in legumes (Elkoca et al. 2008). *Bacillus subtilis* is of particular interest since it is known to positively influence plant growth, vitality, and the ability of the plant to cope with pathogens often resulting in higher yield (Elkoca et al. 2010). Inoculation with co-inoculants of these superior strains of *B. japonicum* together with *B. subtilis* would therefore have the potential to improve crop yields due by providing a more balanced nutrition to plants as compared to single-strain inoculants (Tsigie et al. 2012). Bashan et al. (2004) emphasized the importance of biological nitrogen fixation for biomass increase in later developmental stages

These data suggested that the strains of *Bradyrhizobium japonicum* and *Bacillus* sp. rhizobacteria were act as effective bioinoculant, together with the reports indicating IAA production and phosphate solubilization in stationary stage of culture.

4. CONCLUSIONS

Only R1 and B1 strains did not show the ability of the solubilization of calcium phosphates.

The highest IAA production was observed by *Bacillus* sp. strain B4.

Inoculation of soybean had greater impact on the increase in height and dry weight of the aboveground plant parts.

On most inoculation treatments, co-inoculation resulted in significantly greater length and dry weight of the aboveground parts, compared to treatments where the same bacterial strains were applied as single component inoculants.

Maximum height of the aboveground parts was observed in the plants inoculated with a mixture of strains B3 + R1 and B2 + R2, while using a mixture of strains B3 + R2 and B4 + R2 resulted in highest dry weight.

Significantly longer root system compared to control was only observed after treatment with the strain B4.

The highest root dry weight was observed in the plants co-inoculated with a strains B3 + R2.

Co-inoculation with rhizobia and plant growth promoting rhizobacteria are a next subject for research as they can influence the efficacy of rhizobia. Identification and possible manipulation of plant relationships with PGPR has been considered a basic strategy of modern agriculture. Plant growth promoting rhizobacteria, offers an attractive way to replace chemical fertilizers, supplements and pesticides.

REFERENCES

Anwar, MS, Siddique, MT, Verma, A, Rao, YR, Nailwal, T, Ansari, MW & Pande, V 2014, 'Multitrait plant growth promoting (PGP) rhizobacterial isolates from *Brassica juncea* rhizosphere: Keratin degradation and growth promotion', *Communicative & Integrative Biology*, vol. 7, e27683.

Avice, JC, Larmure, A, Ourry, A, Prudent, M, Voisin, AS & Salon, C 2011, 'PlantN fluxes andmodulation by nitrogen, heat and water stresses: a review based on comparison of legumes and non legume plants', *In Abiotic Stress in Plants: Mechanisms and Adaptations Eds. A Shanker and B Venkateswarlu. INTECH*. doi: 10.5772/23474.

Bashan, Y, Holguin, G & de-Bashan, L 2004, '*Azospirillum*-plant relationships: physiological, molecular, agricultural, and environmental advances' *Can J Microbiol*, vol. 50, pp. 521–77.

Bashan, Y & de-Bashan, LE 2010, 'How the plant growth-promoting bacterium *Azospirillum* promotes plant growth – a critical assessment', *Adv Agron*, vol. 108, pp. 77–136.

Chaparro, JM, Sheflin, AM, Manter, DK & Vivanco, JM 2012, 'Manipulating the soil microbiometo increase soil health and plant fertility', *Biol Fertil Soils*; vol. 48, pp. 489–99.

Das, AJ, Kumar, M & Kumar R 2013, 'Plant Growth Promoting Rhizobacteria (PGPR): An Alternative of Chemical Fertilizer for Sustainable, Environment Friendly Agriculture', *Res J Agric Fores Sci*, vol. 1, pp. 21-3.

Dileep Kumar, SB, Berggren, I & Martensson, AM 2001, 'Potential for improving pea production by co- inoculation with fluorescent *Pseudomonas* and *Rhizobium'*, *Plant Soil*, vol. 229, no. 1, pp. 25-34.

Dodd, IC, Zinovkina, NY, Safronova, VI & Belimov, AA 2010, 'Rhizobacterial mediation of plant hormone status', *Ann Appl Biol*, vol. 157, pp. 361–379.

Dubey, SK 1996, 'Combined effect of *Bradyrhizobium japonicum* and phosphate-solubilizing *Pseudomonas* striata on nodulation, yield attributes and yield of rainfed soybean (*Glycine max*) under different sources of phosphorus in Vertisols', *Ind J Microbiol*, vol. 33, pp. 61–65.

Elkoca, E, Kantar, F & Sahin, F 2008, 'Influence of nitrogen fixing and phosphorus solubilising bacteria on the nodulation, plant growth, and yield of chickpea', *J Plant Nutr*, vol. 31, pp. 157–171.

Elkoca, E, Turan, N & Figen Donmez, M 2010, 'Effects of single, dual and triple inoculations with *Bacillus subtilis*, *Bacillus megaterium* and *Rhizobium leguminosarum* bv. *Phaseoli* on nodulation, nutrient uptake, yield and yield parameters of common bean (*Phaseolus vulgaris* l. cv. 'elkoca-05')', *J Plant Nutr*, vol. 33, no. 14, pp. 2104–2119.

Ferreira, MC & Hungria, M 2002, 'Recovery of soybean inoculant strains from uncropped soils in Brazil', *Field Crop Res*, vol. 79, pp. 139–152.

Freitas, ADS, Vieira, CL, Santo,s CERS, Stamford, NP & Lyra MCCP 2007, 'Caracterizacao de rizobios isolados de Jacatupe cultivado em solo salino no Estado de Pernanbuco', *Brasil Bragantia*, vol. 66, pp. 497-504.

Friesen, ML, Porter, SS, Stark, SC, von Wettberg, EJ, Sachs, JL & Martinez-Romero, E 2011, 'Microbially mediated plant functional traits', *Annu Rev Ecol Evol Syst*, vol. 42, pp. 23–46.

Fuhrmann, JJ 1993, 'Population diversity grouping of soybean bradyrhizobia', *in Advances in Agronomy* Vol 50. New York: Academic Press, pp. 67-105.

Glick, B 1995, 'The enhancement of plant growth by free-living bacteria', *Can J Microbiol*, vol. 41, pp. 109–117.

Glick, B, Cheng, Z, Czarny, J & Duan, J 2007, 'Promotion of plant growth by ACC deaminasec-ontaining soil bacteria' *Eur J Plant Pathol*, vol. 119, pp. 329–39.

Glickmann, E & Dessaux, Y 1995, 'A Critical Examination of the Specificity of the Salkowski Reagent for Indolic Compounds Produced by Phytopathogenic Bacteria', *Applied and Environmental Microbiology*, vol. 61, no. 2, pp. 793–796.

Goh, CH, Veliz Vallejos, DF, Nicotra AB & Mathesius U 2013, 'The impact of beneficial plant-associated microbes on plant phenotypic plasticity', *J Chem Ecol*, vol. 39, pp. 826–839.

Goldstein, AH & Krishnaraj, PU 2007, 'Phosphate solubilizing microorganisms vs. phosphate mobilizing microorganisms: What separates a phenotype from a trait?' *In: Velazquez E, Rodríguez-Barrueco C, eds. First International Meeting on Microbial Phosphate Solubilization. Springer*; pp. 203-213.

Guimaraes, AP, Morais, RF, Urquiaga, S, Boddey, RM & Alves, BJR 2008, '*Bradyrhizobium* strain and the 15N natural abundance quantification of biological N2 fixation in soybean', *Sci Agric*, vol. 65, pp. 516–524.

Hunter, WJ 1994, 'Increased nodulation of soybean by a strain of *Bradyrhizobium japonicum* with altered tryptophan metabolism', *Lett Appl Microbiol*, vol. 18, pp. 340-342.

Masciarelli, O, Llanes, A & Luna, V 2014, 'A new PGPR co-inoculated with *Bradyrhizobium japonicum* enhances soybean nodulation', *Microbiological Research*, vol. 169, no. 7-8, pp. 609–615.

Medeot, DB, Paulucci, NS, Albornoz, AI, Fumero, MV, Bueno, MA, Garcia, MB, Woelke, MR, Okon, Y & Dardanelli, MS 2010, 'Plant growth promoting rhizobacteria improving the legume-rhizobia symbiosis', *In: Khan MS, Zaidi A, Musarrat J (eds) Microbes for legume improvement*. Springer-Verlag/Wien, Berlin, pp 473–494.

Pikovskaya, RI 1948, 'Mobilization of phosphorous in soil connection with the vital activity of some microbial species', *Microbiologia*, vol. 17, pp. 362-70.

Prudent, M, Salon, C, Souleimanov, A, Emery, RJN & Smith, DL. 2015, 'Soybean is less impacted by water stress using *Bradyrhizobium japonicum* and thuricin-17 from *Bacillus thuringiensis*', *Agronomy for Sustainable Development*, vol. 35, no. 2, pp. 749-757.

Raeipour, L & Aliasgharzadeh, N 2004, 'Interaction of phosphate solubilizing bacteria and *Bradyrhizobium japonicum* on yield and absorption of some nutrients in soybean', *J Agric Know,* vol. 4, no. 15, pp. 141- 156.

Richardson, AE 2000, 'Prospects for using soil microrganisms to improve the acquisition of phosphorus by plants', *Australian Journal of Plant Physiology*, vol. 28, pp. 897-906.

Rodriguez, H & Fraga, R 1999, 'Phosphate solubilizing bacteria and their role in plant growth promotion', *Biotechnology Advances*, vol. 17, pp. 319-339.

Sakthivel, U & Karthikeyan, B 2012, 'Isolation and Characterization of Plant Growth Promoting Rhizobateria (PGPR) from rhizosphere of *Cloeus forskohlii* grown soil', *Int J Rec Sci Res,* vol. 2, pp. 288-96.

Tsigie, A, Tilak, KVBR & Anil KS 2012, 'Field response of legumes to inoculation with plant growth-promoting rhizobacteria', *Biol Fertil Soils*, vol. 47, pp. 971–974.

Vassilev, N, Medina, A, Azcon, R & Vassileva, M 2006, 'Microbial solubilization of rock phosphate on media containing agro-industrial wastes and effect of the resulting produscts on plant growth and P uptake', *Plant and Soil*, vol. 287, pp. 77-84.

Wasule, D, Wadyalkar, S & Buldeo, A 2007, 'Effect of phosphate solubilizing bacteria on role of *Rhizobium* on nodulation by soybean' *In: Velazquez E, Rodriguez-BarruecoC, editors. First international meeting on microbial phosphate solubilization*, pp. 139–42.

Zaidi, A, Khan, MS, Ahemad, M & Oves, M 2009, 'Plant growth promotion by phosphate solubilizing bacteria', *Acta Microbiol Immunol Hung*, vol. 56, pp. 263-84.